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EFFECT OF *CYMBOPOGON CITRATUS* TERPENOIDS AGAINST BACTERIAL PATHOGENS CAUSING BOVINE MASTITIS

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ABSTRACT

Phytochemical analysis of *Cymbopogon citratus* revealed presence of bioactive compounds such as alkaloids, flavonoids, saponins, tannins, phenols, terpenoids etc. Each of the bioactive compounds were estimated and isolated separately by solvent-solvent extraction for methanolic and aqueous extracts of *Cymbopogon citratus*. Saponins content was higher in methanolic extracts of *Cymbopogon citratus* (23.64%). This was followed by terpenoids with highest of all in methanolic extracts (10.2%). Alkaloids were the least present. Isolated bioactive compounds from crude extracts were tested for antibacterial activity against pathogens causing Bovine mastitis such as *Escherichia coli, Streptococcus uberi, Staphylococcus aureus,* and Coagulase-negative *staphylococcus aureus*. Terpenoids from aqueous extracts of Cymbopogon citratus have shown antibacterial activity with highest zone of inhibition against *E. coli* (17mm) and least against *E. coli* and *S. uberis* (12mm) by methanolic extracts. Hence terpenoids is important bioactive compounds. Aqueous extracts revealed significant activity as compared to methanolic extracts.

KEYWORDS: ***Cymbopogon citratus*, Terpenoids, Bovine mastitis, Antibacterial activity, Phytochemical analysis, Methanol extracts, Aqueous extracts.**

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INTRODUCTION

The World Health Organization (WHO) noted that the majority of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants are widely used as medicine and constitute a major source of natural organic compounds[22]. Some medicinal plants have been used for a wide variety of purposes such as food preservation, pharmaceutical, alternative medicine, and natural therapies for many thousands of years. It is generally considered that compounds produced naturally, rather than synthetically, will be biodegraded more easily and therefore be more environmentally acceptable. This study is an effort towards finding bioactive compounds from *Cymbopogon citratu*s. Bovine Mastitis is an intramammary infection which is most common among the dairy cattle and reduces milk yield, producer's profits and milk product quality. Microbiological causes of mastitis are many and multiple factors involved in the management, housing, milking should be considered and continues to be the costliest disease in the dairy industry all over the world [1].The repeated use of antibiotics to treat Mastitis for a long period may cause multidrug resistivity in causative organisms which requires high doses of antibiotics may leads to accumulation of large amount of antibiotics in milk and its products, again a potential hazard [2].

About the plant

It is a tall perennial grass commonly called as Lemongrass native to India and tropical Asia. It is widely used as an herb in Asian cuisine. It has a subtle citrus flavour and can be dried and powdered or used fresh.

MATERIAL AND METHODS

All the solvents and reagents used in the study were analogue grade sourced from Hi media.

Collection and Extraction of plant material

The plant was collected in the month of March-2011 from Acharya Institute of technology campus, soladevanhalli, Bangalore. The plant with leaves was rinsed with sterilized water and leaves were removed and separated. The leaves were air dried for 3 weeks and then crushed with mortar and pestle and kept in air tight glass container at 4^0C until further use [8,9].

Preparation of crude extracts

Aqueous extract was prepared by using 500g of crushed leaves and 500 ml of distilled water in soxhlet apparatus and the apparatus was allowed to run for 10 hours. Similarly the methanol Extract was prepared [16].

Bacterial strains

Bacterial strains used in this study were isolated from clinical cases of Bovine mastitis namely *Staphylococcus aureus*, *Streptococcus uberis*, *Escherichia coli* and coagulase negative *Staphylococcus aureus*. All the strains were confirmed by cultural and biochemical studies [6] and maintained in nutrient agar slants at 4ºC for further use.

Antibacterial activity

The antibacterial assay of aqueous and methanolic extracts was performed by agar disc diffusion
method [8,9]. The molten Mueller Hinton agar was inoculated with 100µl of the inoculums (1*106 CFU/ml) and poured into the petriplate (HI media). For agar disc diffusion method, the disc (0.7cm), (HI media) was saturated with 100µl of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated overnight at 37ºC. Microbial growth was determined by measuring the diameter of the zone of inhibition of each bacterial strain [22].

Phytochemical analysis

Phytochemical analysis for major phytoconstituents of the plant extracts was undertaken using standard qualitative methods as described by various authors [12,14]. The

plants extracts were screened for the presence of biologically active compounds like glycosides, alkaloids, Phenolics,
tannins, flavonoids, saponins and steroids

Estimation and extraction of phytocompounds

Alkaloids, Flavonoids, Saponins, Phenols, and Tannins were isolated as follows

Alkaloids

Isolated crude sample is extracted with solvent ether and alcohol mixture (4:1) and ammonia solution (5% v/v). To, this 1N H2SO4 followed by 0.5N H2SO4 and alcohol mixture (3:1) is added and the acid layers are separated until the aqueous layer is colorless. This acid layer is then washed with chloroform. Further this chloroform layer is washed with acid alcohol mixture. This layer is then added with 5% v/v ammonia solution in excess. This is then extracted with chloroform and washed with water. The chloroform layer is filtered through a layer of anhydrous sodium sulfate in pre-weighed beaker. The chloroform is allowed to evaporate followed by addition of alcohol which is then dried at 105 □C in hot air oven alkaloids been left in the beaker. The beaker in then weighed to know the content alkaloids isolated.

Flavonoids

Isolated crude sample is dissolved in water washed with hexane to remove oil content. The aqueous layer is washed with chloroform followed by warming the aqueous layer. This warmed aqueous layer is extracted with ethyl-acetate into pre-weighed beaker. The ethylacetate extracted layer is concentrated and dried at 105 □C in hot air oven and the beaker is weighed again. [21,15].

Saponins

Isolated crude sample is extracted with 90% methanol and further concentrated to more than half of the original. This concentrated extract is then extracted with petroleum ether followed by chloroform. The obtained aqueous layer is washed with 90% methanol and again allowed to concentrate. This is then added into pre-weighed beaker containing acetone drop by drop to form saponin precipitates. This is then filtered through pre-weighed filter paper. The pre-weighed beaker and filter paper are then allowed to dry at 105 □C in hot air oven.

Tannins

The material was extracted with mixture of distilled water and 8% Sodium carbonate in a boiling flask under reflux for two hours having used a liquor/ crude extract ratio 15:1. This was repeated again and again to produce more of tannins. After extraction, the material was filtered under vacuum using a Büchner funnel. Finally the filtrate in pre-weighed beaker was dried in hot air oven at 105 □C [20,10] .

Phenolic compounds

Isolated crude sample was extracted with 20 mL of the extracting ethanol in a conical flask. Conical flask was covered with parafilm and aluminium foil to prevent light exposure. The mixture was shaken at constant rate using a water bath shaker for 2 hrs at 50 □C. The ethanol extracted was then filtered through a Whatman No. 1 filter paper into a pre-weighed beaker, and the filtrate was evaporated at 105 □C[20,10].

Terpenoids

Terpenoids were isolated in the form of essential oils. Isolated crude sample was extracted with solvent- hexane. This is then washed with alcohol and the hexane layer is evaporated in water bath to concentrate and then evaporated in hot air oven at 105 □C

RESULTS AND DISCUSSION

Crude methanolic and aqueous extracts of *Cymbopogon citratus* were isolated and phytochemical analysis was performed (Table 1).

Table 1
Methanolic and aqueous extracts of Cymbopogon citrates

Bioactive compounds	*Cymbopogon citrates*	
	Methanol	**Water**
Steroids	-	-
Terpenoids	+	+
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Phenolic compounds	+	+

Most of the secondary metabolites were identified in the polar extracts. Terpenoids Alkaloids, Flavonoids, Saponins, Tannins, Phenolic compounds were present in both methanol and water extracts. Steroids were absent in both solvent extracts (Table I). Bioactive compounds present in samples were isolated separately and the content of each was found by Content. Content (%) = (Weight of phtyocompounds)/ (weight of crude extract) * 100 Content of saponin was found to be highest in both the extracts with the highest of all in methanolic 23.64%. This was followed by Terpenoids 10.12%, Phenolic compounds 0.789%, Tannins 0.982%, Flavonoids 1.012% and Alkaloids 0.187%. In aqueous extract Saponins was found to be highest 23.31% and followed by Alkoilds 0.16%, Flavonoids 2.1221%, Tanins 0.893%, Phenolic compound 0.834% and Terpins 8.3% (Table II, Fig-1) and antibacterial activity was performed for these isolated compounds (Fig-2).

Table 2
Content analysis of methanolic and aqueous extracts of Cymbopogon citrates

PHYTOCOMPOUNDS	METHANOL(%) WATER(%)	
Alkaloids	0.187	0.16
Flavonoids	1.012	2.121
Saponins	23.64	23.31
Tannins	0.982	0.893
Phenolic compounds	0.789	0.834
Terpenoids	10.12	8.3

Methanolic extract of Terpenoids showed zone of inhibition 12mm,13mm,12mm and 13mm for S uberis, S aureus, E coli and Coagulase-negative(CONS) and 13mm, 15mm, 17mm, and 13mm for water extract respectively(Table III).No other compounds both methanol and water extract exhibits antibacterial activity except Terpenoids(Fig-3).

Figure 1
Content of phytocompounds in crude methanolic and aqueous extracts of Cymbopogon citrates

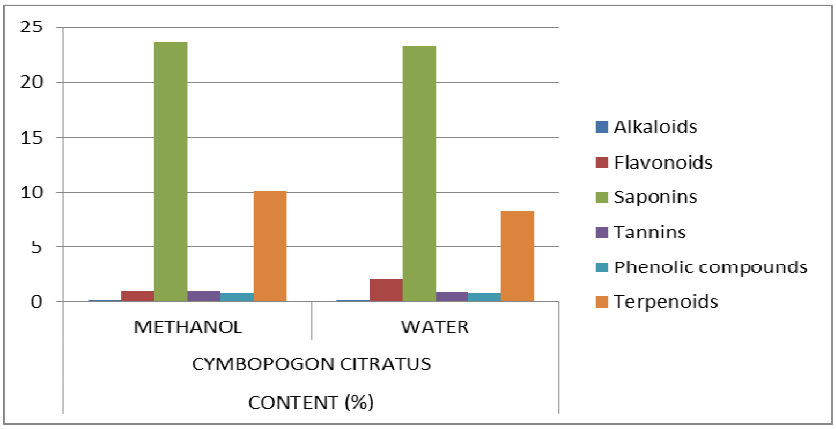


Figure 2
Zone of inhibition of specific bioactive compounds against causative organisms of bovine mastitis

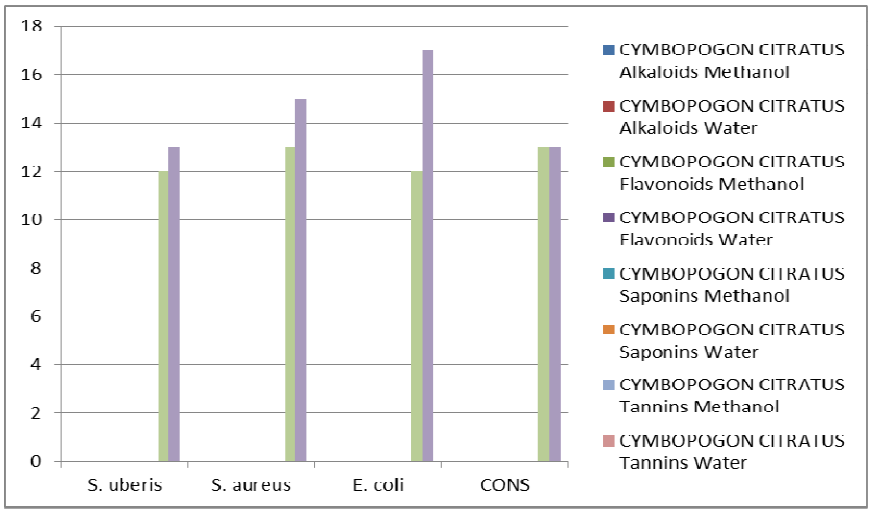
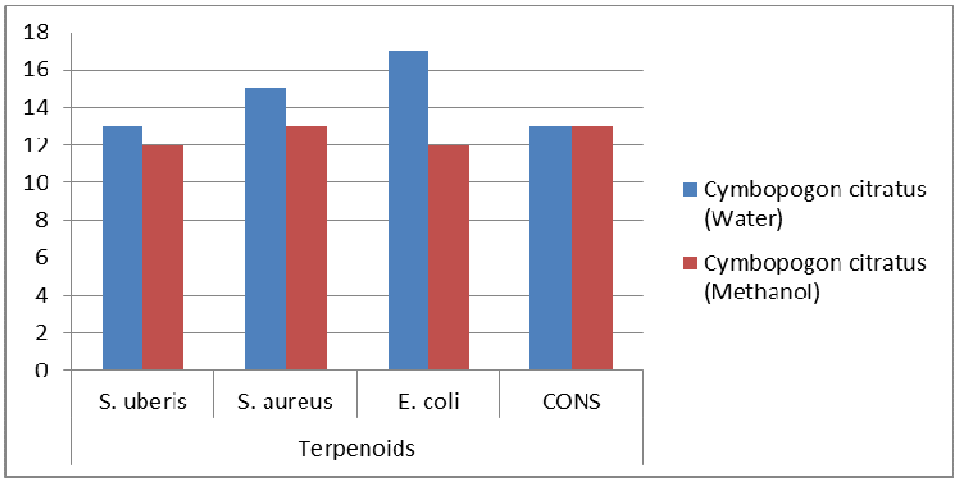


Table-3
Antibacterial activity of different phytocompounds of Cymbopogon citratus (in mm)

Causative organisms	Alkaloids		Flavanoids		Saponins		Tannins		Terpenoids	
	M	W	M	W	M	W	M	W	M	W
S.uberis	0	0	0	0	0	0	0	0	12	13
S.aureus	0	0	0	0	0	0	0	0	13	15
E.coli	0	0	0	0	0	0	0	0	12	17
CONS	0	0	0	0	0	0	0	0	13	13

- ***M – Methanol***
- ***W- Water***

Figure 3
Zone of inhibition v/s Terpenoids



CONCLUSION

The methanol and water extracts of Terpenoids from *Cymbopogon citratus* have potential antibacterial properties. The water extracts of Terpenoids exhibits maximum inhibition zone against causative organisms of Bovine mastitis. Further development of vital drug from Terpenoids is very essential in control of Bovine mastitis, quality of milk and economy of the dairy industry.

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